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Comparison of Characteristics Between Patients With GB Virus C/Hepatitis G Virus (GBV-C/HGV) RNA and Those With GBV-C/HGV E2-Antibody in Patients With Hemophilia

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We compared characteristics of patients with GB virus C/hepatitis G virus (GBV-C/HGV) RNA to those of patients with GBV-C/HGV E2-antibody. GBV-C/HGV RNA and GBV-C/HGV antibody were assayed in 83 persons with hemophilia using a reverse transcription-polymerase chain reaction and an enzyme-linked immunosorbent assay, respectively. GBV-C/HGV RNA was detected in 19 (22.9%) patients and GBV-C/HGV antibody was detected in 17 (20.5%). The background characteristics between the patient groups did not differ with respect to age, severity of hemophilia based on the frequency of use of blood product, and both the initial age at the first use and years since the first use of blood products. There were no differences in coinfection with hepatitis C virus (HCV) and/or human immunodeficiency virus, except that infection with HCV subtype 1a was more prevalent in patients with GBV-C/HGV RNA ($P = 0.0229$). Human lymphocyte antigen (HLA) typing was conducted in 18 patients with GBV-C/HGV RNA and 15 patients with GBV-C/HGV E2-antibody; 13 of the patients with GBV-C/HGV antibody had either HLA DQ7, DR15, or DR8, whereas only 4 of the patients with GBV-C/HGV RNA did ($P < 0.001$). It is concluded that the presumed age at the time of GBV-C/HGV infection, the frequency of exposure to GBV-C/HGV, and the time since the GBV-C/HGV infection were not associated with recovery from infection with GBV-C/HGV. Coinfection with HCV subtype 1a may be related to persistent GBV-C/HGV viremia, whereas HLA DQ7, DR15, or DR8 may be related to the clearance of GBV-C/HGV after infection. ***J. Med. Virol. 60:34–38, 2000.***

KEY WORDS: GBV-C/HGV RNA; GBV-C/HGV E2-antibody; hemophilia; HLA

INTRODUCTION

Recently, a new Flaviviridae-associated virus, GB virus C (GBV-C), was isolated [Simons et al., 1995]. Hepatitis G virus (HGV) has also been reported [Linnen et al., 1996], and these two viruses are considered to be isolates of the same virus [Zuckerman, 1996]. In addition, the ability to detect GBV-C/HGV E2-antibody has been established [Dille et al., 1997; Tacke et al., 1997]. The presence of GBV-C/HGV E2-antibody in the serum represents past infection with GBV-C/HGV, whereas the absence of GBV-C/HGV RNA correlated with clearance of the virus [Thomas et al., 1998]. However, some patients do not develop an anti-E2 response [Dille et al., 1997] and remain infected with GBV-C/HGV for years [Masuko et al., 1996]. What induces clearance of GBV-C/HGV and production of E2-antibody and what factors are important for the eradication of GBV-C/HGV are not known.

In this study, Japanese patients with GBV-C/HGV RNA were compared with those with GBV-C/HGV E2-antibody.

PATIENTS AND METHODS

Patients

All patients with a bleeding disorder attending Nagoya University Hospital as outpatients and who were admitted to the hospital during 1996 were studied. Of 83 patients (81 men and 2 women; mean age, 31.3 years; range, 9–76 years), 58 had hemophilia A, 22 had hemophilia B, 1 had von Willebrand disease, 1 had afibrinogenemia, and 1 had athrombia (two female patients had afibrinogenemia and athrombia, respectively). GBV-C/HGV RNA and GBV-C/HGV E2-antibody were measured in all patients. Patients with

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TABLE I. Characteristics of Patients With GBV-C/HGV RNA and of those with GBV-C/HGV E2-Antibody

	GBV-C/HGV RNA+ /E2-antibody–	GBV-C/HGV RNA– /E2-antibody+
Number	19	17
Hemophilia (A/B)	13/6	11/6
Mean age, years (range)	28.6 (12–47)	33.5 (12–50)
Severity of bleeding disorder (severe/moderate/mild)	15/1/3	11/2/4
Age at the first use of blood products, years (range)	6.4 (0.5–25)	7.9 (0.3–35)
Time since the first use of blood products, years (range)	20.3 (6–42)	23.9 (8–38)

GBV-C, GB virus C; HGV, hepatitis G virus.

GBV-C/HGV RNA were compared with those who had GBV-C/HGV E2-antibody with respect to baseline characteristics, including age, severity of bleeding disorder based on the frequency of use of blood products, the initial age at the first use and years since the first use of blood products, the prevalence of coinfection with hepatitis C virus (HCV) and/or human immunodeficiency virus (HIV) and the respective viral load, and human leukocyte antigen (HLA) typing. Bleeding disorder severity was defined as follows: patients who required blood products at least once a week were defined as "severe," patients who required blood products at least once a month were defined as "moderate," and patients whose frequency of blood product use was less than once per month were considered "mild."

Detection of GBV-C/HGV RNA and E2-Antibody

GBV-C/HGV RNA was measured by reverse transcription-polymerase chain reaction (RT-PCR) with nested primers deduced from conserved blocks in the 5′-untranslated region [Toyoda et al., 1998]. The first round was performed with antisense primer #G75 with a sequence of 5′-CCTATTGGTCAAGAGAGACAT-3′ and sense primer #G58 with a sequence of 5′-CAGGGTTGGTAGGTCGTAAATCC-3′ for 35 cycles (94°C, 30 sec; 55°C, 30 sec; 72°C, 60 sec). The second round was carried out with nested primers, sense primer #G134 with a sequence of 5′-GGTCAYCYTGGTAGCCACTATAGG-3′ and antisense primer #G131 with a sequence of 5′-AAGAGAGACATTGWAGGGCGACGT-3′, for 25 cycles (94°C, 30 sec; 55°C, 30 sec; 72°C, 60 sec). GBV-C/HGV E2-antibody was measured via an enzyme-linked immunosorbent assay (ELISA) [Tacke et al., 1997] (Enzymun-Test Anti-HGenv kit, Boehringer Mannheim, Mannheim, Germany).

Evaluation of Coinfection With HCV and HIV

HCV coinfection was determined using HCV RNA detection by nested RT-PCR [Okamoto et al., 1990]. In patients with detectable HCV RNA, serum HCV RNA concentrations were measured using a branched DNA (bDNA) probe assay, via a bDNA signal-amplification assay kit (Quantiplex HCV-RNA, version 2.0, Chiron Corp., Emeryville, CA) [Detmer et al., 1996]. The five common genotypes of HCV according to Simmonds' classification (genotypes 1a, 1b, 2a, 2b and 3a) [Simmonds et al., 1994], were determined by RT-PCR with genotype-specific primers deduced from the HCV core gene using a second-generation method of genotyping [Okamoto et al., 1996]. HIV coinfection was determined by anti-HIV1 antibody detection by a particle agglutination assay (SERODIA-HIV, Fuji Rebio, Tokyo, Japan). In patients with anti-HIV1 antibody, HIV RNA concentrations were measured using the Amplicore HIV Monitor test (Roche Molecular Systems, Somerville, NJ).

HLA Typing

HLA-A, B, and Cw typing was carried out using standard lymphocyte microcytotoxicity tests with a panel of sera [Terasaki and McClelland, 1964] in the HLA section of the Japanese Red Cross Aichi Blood Center after T-lymphocyte enrichment was achieved using selected HLA-Class I Immuno-beads. HLA-DR and DQ serologic typing was undertaken using the same technique after B-lymphocyte enrichment with both HLA-Class II Immuno-beads and PANB Immuno-beads. All typing reagents were prepared in the laboratory. Cells not reacting with the typing sera used to identify the two allelic phenotypes were denoted as blank. HLA-DQA1, DQB1, DRB1, and DPB1 genetic typing was undertaken in the same patients. Genomic DNA was isolated from 10 ml of peripheral blood collected in an ACD anticoagulant, and HLA-DQA1, DQB1, and DRB1 typing was carried out using modified PCR-restriction length polymorphism (RFLP) [Ota et al., 1991, 1992; Nomura et al., 1994]. DPB1 typing was carried out by the PCR-RFLP [Maeda et al., 1990].

RESULTS

GBV-C/HGV RNA was detected in 19 (22.9%) of 83 patients and GBV-C/HGV antibody was detected in 17 (20.5%). No patient had both GBV-C/HGV RNA and antibody. No differences in the background characteristics existed between the two patient groups (Table I). The frequency, age at the first use, and time since the first use of blood products was similar between these two groups.

The prevalence of coinfection with HCV was 94.7% (18 of 19 patients) in patients with GBV-C/HGV RNA and was 94.1% (16 of 17 patients) in patients with an GBV-C/HGV E2-antibody. The prevalence of coinfection with HIV was 57.9% (11 of 19 patients) in those with GBV-C/HGV RNA and 35.3% (6 of 17 patients) in those with an GBV-C/HGV E2-antibody. There were no significant differences in prevalence between these two groups respectively. The viral load of coinfecting with HCV and/or HIV

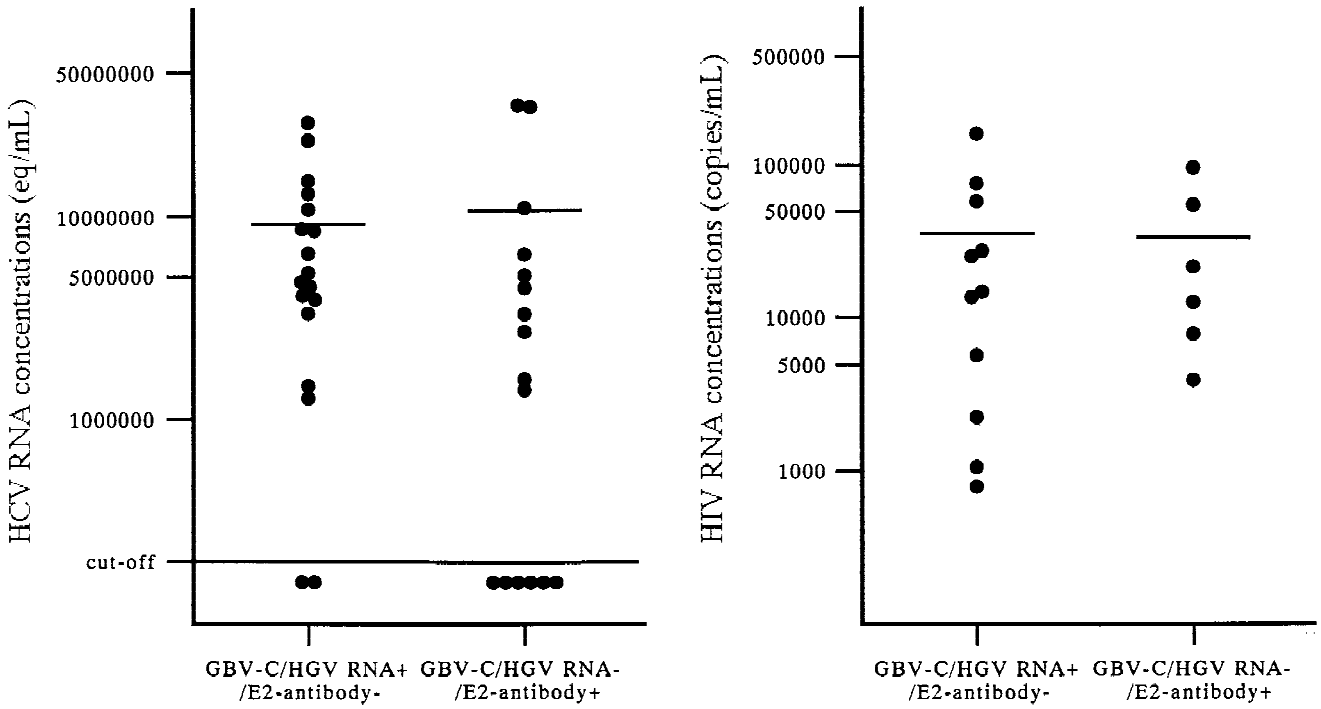

Fig. 1. The viral concentrations of coinfected hepatitis C virus (HCV) (left) and human immunodeficiency virus (HIV) (right). HCV RNA concentration was $9.09 \pm 7.88 \times 10^6$ eq/ml in patients with GB virus C/hepatitis G virus (GBV-C/HGV) RNA and was $10.53 \pm 12.94 \times 10^6$ eq/ml in those with GBV-C/HGV E2-antibody. HIV RNA concentration was $3.53 \pm 4.81 \times 10^4$ copies/ml in patients with GBV-C/HGV RNA and was $3.33 \pm 3.66 \times 10^4$ copies/ml in those with GBV-C/HGV E2-antibody. (HCV RNA was undetectable by bDNA probe assay in 2 of 18 (10.5%) HCV-infected patients with GBV-C/HGV RNA and in 6 of 17 (35.3%) those with GBV-C/HGV E2-antibody.)

was similar in both groups (Fig. 1). HCV RNA concentration was $9.09 \pm 7.88 \times 10^6$ eq/ml in patients with GBV-C/HGV RNA vs. $10.53 \pm 12.94 \times 10^6$ eq/ml in those with GBV-C/HGV E2-antibody. HCV RNA was not detectable by bDNA probe assay in 2 of 18 (10.5%) HCV-infected patients with GBV-C/HGV RNA and in 6 of 17 (35.3%) those with GBV-C/HGV E2-antibody. HIV RNA concentration was $3.53 \pm 4.81 \times 10^4$ copies/ml in patients with GBV-C/HGV RNA vs. $3.33 \pm 3.66 \times 10^4$ copies/ml in those with GBV-C/HGV E2-antibody.

The distribution of HCV subtype coinfection did not differ between the groups, except for HCV subtype 1a, which was more prevalent in patients with GBV-C/HGV RNA ($P = .0229$, Table II).

HLA typing was carried out in 18 patients with detectable GBV-C/HGV RNA and in 15 patients with an GBV-C/HGV E2-antibody. HLA-serologic typing revealed a higher incidence of HLA DQ7, DR15, or DR8 in patients with GBV-C/HGV antibody, but this did not achieve statistical significance when the P value was corrected (DQ7, $P = .0338$, $Pc = .135$; DR15, $P = .0287$, $Pc = .258$; DR8, $P = .0533$, $Pc = .480$). However, 13 of 15 patients (86.7%) with GBV-C/HGV antibody had either HLA DQ7, DR15, or DR8 (1 patient had HLA DQ7, DR15, and DR8, and 2 had both HLA DR15 and DR 8), whereas only 4 of 18 patients (22.2%) with GBV-C/HGV RNA had ($P < .001$, Table III). Of note, the prevalence of HLA DQ7, DR15, and DR8 in the Japanese population is 11.62%, 15.67%, and 10.72%, respectively, according to the 10th HLA Workshop of Japan, 1991. Using HLA class II DNA typing, no significant differences were found between patients with GBV-C/HGV RNA and those with GBV-C/HGV E2-antibody, except that DRB1*1502 tended to be more prevalent in patients with GBV-C/HGV E2-antibody ($P = .0171$, $Pc = .289$).

TABLE II. Subtype of HCV Coinfection in Patients With GBV-C/HGV RNA and in Those With GBV-C/HGV E2-Antibody

Subtype of HCV	GBV-C/HGV RNA+/E2-antibody–	GBV-C/HGV RNA–/E2-antibody+
1a	8 (44.4%)	2 (12.5%)
1b	4 (22.2%)	6 (37.5%)
2a	1 (5.6%)	2 (12.5%)
2b	1 (5.6%)	1 (6.3%)
3a	3 (16.6%)	5 (31.2%)
Mixed	1 (5.6%)	0

HCV, hepatitis C virus; GBV-C, GB virus C; HGV, hepatitis G virus.

DISCUSSION

Recent studies have shown that unlike HCV, GBV-C/HGV lacks antigenic drift and does not have hypervariable regions as found in both HCV and HIV [Nakao et al., 1997; Kato et al., 1998]. GBV-C/HGV, therefore, may employ a strategy for viral persistence that is different from immune escape [Nakao et al., 1997].

In the present study, patients with hemophilia were studied because data regarding their use of blood products were readily available. The age at first blood prod-

TABLE III. HLA Typing of Patients With GBV-C/HGV RNA and of Those With GBV-C/HGV E2-Antibody

	GBV-C/HGV RNA+/E2-antibody–	GBV-C/HGV RNA–/E2-antibody+
HLA typing		
Patients with either HLA DQ7, DR15, or DR8	4	13
Patients without HLA DQ7, DR15, or DR8	14	2

HLA, human lymphocyte antigen; GBV-C, GB virus C; HGV, hepatitis G virus.

uct use was assumed to represent the presumed age at the first exposure to GBV-C/HGV and number of years since the first blood product use to represent the presumed duration of GBV-C/HGV carriage. The severity of hemophilia as evidenced by the frequency of blood products use represented the presumed frequency of the exposure to GBV-C/HGV.

The prevalence of GBV-C/HGV RNA in patients with hemophilia tends to decrease as the length of time from first blood product use increases [Hanley et al., 1998], suggesting clearance of GBV-C/HGV over time. However, a previous report described patients with the loss of GBV-C/HGV RNA with the production of GBV-C/HGV E2-antibody within a few weeks [Tacke et al., 1997], and another study demonstrated patients with persistent GBV-C/HGV viremia for many years [Masuko et al., 1996]. Thus, the period of GBV-C/HGV persistence varies markedly between patients [Thomas et al., 1998]. No difference was observed in the present study in the time since the first use of blood products, which can represent the duration of GBV-C/HGV infection, between patients with GBV-C/HGV RNA and those with GBV-C/HGV E2-antibody.

The two groups did not differ in the presumed age at infection or the presumed frequency of exposure, which are both important factors for chronicity in case of hepatitis B virus infection. In addition, the prevalence of coinfection with HCV or HIV, and their subtypes and concentrations did not differ in the two groups. Thus, coinfection with HCV or HIV did not appear to affect the clearance of GBV-C/HGV. However, there were more patients coinfected with HCV subtype 1a among those with GBV-C/HGV RNA than among those with GBV-C/HGV E2-antibody. Coinfection with HCV subtype 1a may have a suppressive effect on the clearance of GBV-C/HGV RNA.

In the HLA analysis, most patients with E2-antibody had either DQ7, DR15, or DR8, which were not present in those with GBV-C/HGV RNA. HLA class II is associated with the immune system for the eradication of foreign proteins. Therefore, these HLA types, which are common types in the general Japanese population, may be related to the clearance of GBV-C/HGV after infection. Alternatively, the length of the period necessary for the eradication of GBV-C/HGV may differ according to HLA type of the host.

In summary, no difference was observed between the characteristics of patients with GBV-C/HGV RNA and those with GBV-C/HGV E2-antibody. The presumed age at the GBV-C/HGV infection, the presumed duration of the GBV-C/HGV infection, and the presumed frequency of the exposure to GBV-C/HGV were not associated with GBV-C/HGV eradication. Coinfection with HCV subtype 1a may be related to persistent GBV-C/HGV viremia, whereas HLA DQ7, DR15, or DR8 may be related to the clearance of GBV-C/HGV after infection.

REFERENCES

Detmer J, Lagier R, Flynn J, Zayati C, Kolberg J, Collins M, Urdea M, Sanchez-Pescador R. 1996. Accurate quantification of hepatitis C virus (HCV) RNA from all HCV genotypes by using branched-DNA technology. J Clin Microbiol 34:901–907.

Dille BJ, Surowy TK, Gutierrez RA, Coleman PF, Knigge MF, Carrick RJ, Aach RD, Hollinger FB, Stevens CE, Barbosa LH, Nemo GJ, Mosley JW, Dawson GJ, Mushahwar IK. 1997. An ELISA for detection of antibodies to the E2 protein of GB virus C. J Infect Dis 175:458–461.

Hanley JP, Jarvis LM, Hayes PC, Lee AJ, Simmonds P, Ludlam CA. 1998. Patterns of hepatitis G viraemia and liver disease in haemophiliacs previously exposed to non-virus inactivated coagulation factor concentrates. Thromb Haemost 79:291–295.

Kato T, Mizokami M, Nakano T, Orito E, Ohba K-I, Kondo Y, Tanaka Y, Ueda R, Mukaide M, Fujita K, Yasuda K, Iino S. 1998. Heterogeneity in E2 region of GBV-C/hepatitis G virus and hepatitis C virus. J Med Virol 55:109–117.

Linnen J, Wages J Jr, Zhang-Keck Z-Y, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW-K, Young L, Piatak M Jr, Hoover C, Fernandez J, Chen S, Zou J-C, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science 271:505–508.

Maeda M, Uryu N, Murayama N, Ishii H, Ota M, Tsuji K, Inoko H. 1990. A simple and rapid method for HLA-DP genotyping by digestion of PCR-amplified DNA with allele-specific restriction endonucleases. Hum Immunol 27:111–121.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1996. Infection with hepatitis GB virus C in patients on maintenance hemodialysis. N Engl J Med 334:1485–1490.

Nakao H, Okamoto H, Fukuda M, Tsuda F, Mitsui T, Masuko K, Iizuka H, Miyakawa Y, Mayumi M. 1997. Mutation rate of GB virus C/hepatitis G virus over the entire genome and in subgenomic regions. Virology 233:43–50.

Nomura N, Ota M, Tsuji K, Inoko H. 1994. HLA-DQB1 genotyping by a modified PCR-RFLP method combined with group-specific primers. Tissue Antigens 38:53–59.

Okamoto H, Kobata S, Tokita H, Inoue T, Woodfield GD, Holland PV, Al-Knawy BA, Uzunalimoglu O, Miyakawa Y, Mayumi M. 1996. A second-generation method of genotyping hepatitis C virus by the plymerase chain reaction with sense and antisense primers deduced from the core gene. J Virol Methods 57:31–45.

Okamoto H, Okada S, Sugiyama Y, Yotsumoto S, Tanaka T, Yoshizawa H, Tsuda F, Miyakawa Y, Mayumi M. 1990. Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5′-noncoding region. Jpn J Exp Med 60:215–222.

Ota M, Fukushima T, Tsuji K, Inoko H. 1992. HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. Tissue Antigens 39:187–202.

Ota M, Seki T, Nomura N, Sugimura K, Mizuki N, Fukushima T, Tsuji K, Inoko H. 1991. Modified PCR-RFLP method for HLA-DPB1 and -DQA1 genotyping. Tissue Antigens 38:60–71.

Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C,

Brouwer JT, Chan S-W, Chayama K, Chen D-S, Choo Q-L, Colombo M, Cuypers HTM, Date T, Dusheiko GM, Esteban JI, Fay O, Hadziyannis SJ, Han J, Hatzakis A, Holmes EC, Hotta H, Houghton M, Irvine B, Kohara M, Kolberg JA, Kuo G, Lau JYN, Lelie PN, Maertens G, McOmish F, Miyamura T, Mizokami M, Nomoto A, Prince AM, Reesink HW, Rice C, Roggendorf M, Schalm SW, Shikata T, Shimotohno K, Stuyver L, Trepo C, Weiner A, Yap PL, Urdea M. 1994. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 19:1321–1324.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1:564–569.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G, Engel AM. 1997. Detection of antibodies to a putative hepatitis G virus envelope protein. Lancet 349:318–320.

Terasaki PI, McClelland JD. 1964. Microdroplet assay of human serum cytotoxins. Nature 204:998–1000.

Thomas DL, Vlahov D, Alter HJ, Hunt JC, Marshall R, Astemborski J, Nelson KE. 1998. Association of antibody to GB virus C (hepatitis G virus) with viral clearance and protection from reinfection. J Infect Dis 177:539–542.

Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J, Saito H, Okamoto H. 1998. GB virus C/hepatitis G virus isolates in Japanese haemophiliacs and their origins. Thromb Haemost 80:242–245.

Zuckerman AJ. 1996. Alphabet of hepatitis viruses. Lancet 347:558–559.